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(54) A process for the extraction of lycopene and extracts containing it

(57) The present invention relates to a process for the preparation of pure lycopene or of lipophilic extracts containing it from whole fruits of Lycopersicum esculentum and similar species or parts thereof obtainable as by-products of food industry processes. The partially dehydrated fresh material is extracted with aliphatic or aromatic hydrocarbons or water-immiscible solvents in the presence of phospholipids as surfactant and stabilizing agents and the extracts are concentrated to an oil or fractionated to the desired lycopene concentration.

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Description

The present invention relates to a process for the extraction of lycopene and to extracts containing it.

Lycopene is a highly lipophilic procarotenoid mainly present in the species Lycopersicum or in lower amounts in other vegetable or algae species. Lycopene is present in common tomatoes in concentrations ranging from 30 to 100 ppm, on the average at 50 ppm. It is usually present as a secondary metabolite in mammals, where it plays an important role as an antioxidant and free-radicals scavenger, together with other substances such as Vitamin E, Vitamin C and other compounds. At the plasma level, lycopene has proved to be incorporated in low-density lipoproteins in which it decreases the oxidation of cholesterol and of other lipids, thus preventing vascular damages; moreover, an inverse correlation between lycopene blood levels and prostate tumor has recently been proved. The administration of lycopene to humans is therefore important in reducing the risk of tumors and atherosclerosis; hence the need for stable formulations containing lycopene for a preventive chronic treatment in humans.

The present invention relates to a process for the preparation of pure lycopene or lipophilic extracts containing it, starting mainly from whole fruits of Lycopersicum esculentum and similar species or parts thereof obtainable as by-products of industrial food processes. The partially dehydrated fresh material is extracted with aromatic or/and aliphatic hydrocarbons, mainly with n-hexane, or with chlorinated solvents or aliphatic esters, particularly with methylene chloride in the presence of soy lecithin or of other phospholipids as surfactant and stabilizing agents for the procarotenoid, until exhaustion of lycopene in the biomass. According to the invention, fruits of Lycopersicum sp. are homogenised with a knife mill and the resulting semi-liquid mass is heated to temperatures varying from 70 to 100°C, preferably at 90°C, for a time from 15 to 60 minutes, usually' for about 30 minutes. During heating, an abundant precipitate forms, which includes all of the lycopene present in the biomass. The suspension is cooled at 60°C keeping the medium sterile, then centrifuged through fast decanters or filtered through pressure-filters.

The centrifugation or filtration residue, containing about 20% of residual water, can immediately be extracted with stirring or continuously with hydrocarbons, or it can be frozen to preserve it until the extraction. The pressing residue when immediately extracted, is suspended in about 10 vol. of the selected solvent, usually n-hexane, containing soy lecithin at concentrations varying from 0.01 to 0.1%, preferably 0.02%, according to the procedure reported below in the examples. The solvents used in these conditions extract lycopene which generally goes into solution in the first extraction, in a highly selective way, leaving other carotenoids, such as beta-carotene, in the biomass. The extracts are concentrated until the solvent is completely removed, thereby obtaining oily residues containing up to 10% of lycopene.

An economically interesting source for the preparation of lycopene are the by-products of preserved food industry, such as the skins of mature fruits and the not-green fibrous parts. These materials can be quickly dried or better frozen, optionally after removing seeds which are usually present. In case the vegetable material is frozen on the point of the extraction, the biomass must be grinded at temperatures ranging from -25 to -10°C, preferably below -15°C so as to homogenize it; subsequently the residue is extracted according to the procedures reported for the whole fruits; if the vegetable material is dried, before the extraction it is finely grinded and treated with about half part by weight of water to cause the cell lysis thus promoting the extraction; the humid vegetable material is then extracted with aliphatic hydrocarbons or other solvents as described above for the whole fruits. Suitable amounts of phospholipids will be added to assist the extraction and avoid lycopene oxidative degradation. These biomasses also yield, after evaporation of the extraction solvent, lipid extracts containing one to ten percent of lycopene. Pure lycopene can be prepared from these extracts by chromatography using acidic alumina columns and eluting the product with ethyl acetate/hexane mixtures with a variable gradient. Alternatively, lycopene can be prepared extracting the lipophilic extract resulting from the concentration of the extraction solvents by means of partition of the concentrate with CO_2 in supercritical conditions using multisteps columns; as an example, lycopene can be purified using a three-step partitioning column, keeping the pressure constant in a range from 150 to 200 bars, preferably under 180 bars and a segment temperature ranging from 35 to 65°C. In these supercritical conditions a substantial purification of the extract and the recovery of lycopene by crystallization of the residue undistilled during fractionation can be obtained. The products obtained according to the invention can be incorporated in formulations, such as hard- and soft- gelatin capsules, and normal and gastro-resistant tablets. In nutritional preparations for humans for the prevention of atherosclerosis or for the prevention of tumors, lycopene can advantageously be combined with procyanidole polyphenols such as those prepared from Vitis vinifera and Camellia sinensis. When lycopene is used in a substantially pure form, for the preparation of bio-available formulations, the addition of phospholipids as stabilizers and emulsifiers is convenient. In the not purified extracts, phospholipids are already present as they are added during the extraction process.

The following examples further illustrate the invention.

Example I

Preparation of a lycopene-enriched lipophilic extract from Lycopersicum esculentum.

10 kg of mature tomatoes (Lycopersicum esculentum var. Sanmarzano) are homogenized in a knife mill to a semi-fluid mass; the fluid slurry in heated during three hours with flowing steam to 95°C and left in these temperature conditions with slow stirring for about 30 minutes. During heating, a red flocculate separates consisting of denatured vegetable cell structures which tend to precipitate immediately. This suspension is cooled at 60°C and centrifuged; the supernatant not containing lycopene is discarded and the residue is washed in centrifuge with water thereby removing any undesired substances. The biomass still containing about 50% of water is extracted for 3 hours with strong stirring and under mild reflux with 2.5 l of n-hexane containing 0.01% soy lecithin; after separation of the solvent, the residue is re-extracted with a further 2 l of n-hexane in the same operative conditions as in the first extraction. The extraction solvent after filtration and complete decantation is concentrated under vacuum until the solvent is removed completely. 11 g of a brightly red-coloured oily residue containing 0.52 g of lycopene are obtained. This extract can be used as such in the formulations for use in humans.

Example II

Preparation of a lycopene-enriched lipophilic extract starting from dried skins of Lycopersicum esculentum

10 kg of mature tomatoes (Lycopersicum esculentum var. Sanmarzano) are coarsely grinded and filtered by squeezing through a grid which allows the separation of the fruit skins and seeds. The squeezing residue is quickly dehydrated in air stream. About 150 g of a brightly coloured material are obtained, which is separated from the seeds and finely grinded. The grinded residue is treated in a mixer with 150 ml of water and left to stand for about one hour, thereby allowing the swelling of the vegetable material, the breaking of the cells and the membrane porosity. This wet mass is extracted with 800 ml of n-hexane containing 0.01% soy lecithin with stirring under mild reflux; after 3 hours the solvent is removed and the vegetable residue is extracted again twice with 400 ml each of n-hexane. The combined extracts are filtered, decanted, then concentrated under vacuum until the solvent is completely removed. 5.2 g of a brightly red-coloured oily residue containing 4.6% of lycopene are obtained. This extract can be used as such in the formulation for use in humans.

Example III

Preparation of a lycopene-enriched lipophilic extract starting from not-dried skins of Lycopersicum esculentum

The industrial squeezing residue of fresh tomatoes, in conditions similar to those reported in example II, is separated from the juice, washed with water, pressed to remove the residual water as much as possible and frozen. For the extraction, the frozen mass is grinded at a temperature of -20°C and then extracted according to the procedure of example II. 250 g of a frozen biomass yield 4.2 g of a brightly red-coloured oily residue containing 5.6% of lycopene. This extract can be used as such in the formulations for use in humans.

Example IV

Preparation of pure lycopene by extraction from the enriched lipophilic extract

0.5 kg of lycopene oil, prepared according to example I, are extracted in counter-current with carbon dioxide in supercritical conditions, using a three steps column working at different temperatures (50, 40, 34 °C) and under a 180 bars pressure. In these conditions, the purification of the extract is achieved and lycopene is recovered by crystallization of the residue which has not been distilled during the fractionation. 24 g of a pure product are obtained, having the same chemical-physical and spectroscopical characteristics as lycopene.

Example V

Preparation of coated tablets containing the lycopene-enriched lipophilic extract

Lycopene-enriched lipophilic extract	10.00 mg
Lactose	50.60 mg
Pre-gelatinized starch	73.60 mg
Microcrystalline cellulose	49.80 mg
Cross-linked sodium carboxymethylcellulose	19.00 mg
Colloidal silica	19.00 mg
Hydroxypropyl cellulose	5.00 mg
Hydrogenated vegetable oils	2.00 mg
Soy lecithin	1.00 mg
Hydroxypropyl methylcellulose	6.07 mg
Talc	3.96 mg
Triacetin	1.21 mg
Titanium dioxide	0.40 mg
Red iron oxide	0.24 mg
Polyethylene glycol 6000	0.12 mg

Example VI

Preparation of hard-gelatin capsules containing the lycopene-enriched lipophilic extract

Lycopene-enriched lipophilic extract	10.00 mg
Lactose	50.60 mg
Pre-gelatinized starch	73.60 mg
Microcrystalline cellulose	49.80 mg
Cross-linked sodium carboxymethylcellulose	19.00 mg
Colloidal silica	19.00 mg
Hydroxypropyl cellulose	5.00 mg
Hydrogenated vegetable oils	2.00 mg
Soy lecithin	1.00 mg

Example VII

Preparation of hard-gelatin capsules containing lycopene

Lycopene	5.00 mg
Lactose	40.00 mg
Pre-gelatinized starch	53.00 mg
Microcrystalline cellulose	30.00 mg
Colloidal silica	5.00 mg
Hydrogenated vegetable oils	2.00 mg
Cross-linked sodium carboxymethylcellulose	19.00 mg
Hydroxypropyl cellulose	5.00 mg

Example VIII

Preparation of soft-gelatin capsules containing the lycopene-enriched lipophilic extract

Lycopene-enriched lipophilic extract	10.00 mg
Peanut oil	151.00 mg
Partially hydrogenated vegetable oils	80.00 mg
Soy lecithin	1.00 mg

Coating

Gelatin	82.00 mg
Glycerol	40.10 mg
Glycine	1.87 mg
Red iron oxide	1.42 mg

Claims

1. A process for the extraction of lycopene from parts or whole fruits of Lycopersicum esculentum with aromatic or aliphatic hydrocarbons, halohydrocarbons or aliphatic esters, which process is carried out in the presence of phospholipids or soy lecithin in concentrations varying from 0.01 to 0.100 w/v.

2. A process according to claim 1, wherein the extraction is carried out with n-hexane or methylene chloride.

3. A process according to claim 2, wherein the extraction is carried out with n-hexane.

4. A process according to any one of claims 1 to 3, which is carried out in the presence of soy lecithin.

5. A process according to any one of claims 1 to 4, wherein the fruits or parts of Lycopersicum esculentum are previously heated to a temperature from 70 to 100°C for a time varying from 15 to 60 minutes.

6. A process according to any one of the above claims, wherein the resulting lipid extract is further purified by chromatography through an acidic alumina column and elution with ethyl acetate and hexane mixtures.

7. A process according to any one of claims 1 to 5, wherein the resulting lipid extract is further purified by extraction with CO_2 in supercritical conditions.

8. Lipophilic extracts containing 1 to 10% of lycopene obtainable by the processes of claims 1 to 4.

9. Dietetic, food or cosmetic compositions, containing the extracts of claim 8.

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EUROPEAN SEARCH REPORT

Application Number
EP 97 11 1635

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	WO 95 16363 A (MAKHTESHIM CHEM WORKS LTD ;MAKHTESHIM AGAN OF NORTH AMERI (US); ZE)		B01D11/04 A23L1/275 A23L1/212
X	US 3 081 171 A (CORN PRODUCTS COMPANY) * the whole document *	8,9	A61K7/00
X	US 5 180 747 A (MATSUDA YOSHIHISA ET AL) * column 1, line 10 - line 15 * * column 2, line 3 - line 60 *	8,9	
A	DATABASE WPI Section Ch, Week 9532 Derwent Publications Ltd., London, GB; Class D13, AN 95-242717 XP002045928 & JP 07 147 929 A (KAGOME KK) , 13 June 1995 * abstract *		
A	DATABASE WPI Section Ch, Week 9418 Derwent Publications Ltd., London, GB; Class B07, AN 94-148154 XP002045929 & JP 06 093 285 A (HASEGAWA CO LTD) , 5 April 1994 * abstract *		TECHNICAL FIELDS SEARCHED (Int.Cl.6) B01D A23L A61K
X	EP 0 600 544 A (MAKHTESHIM CHEM WORKS LTD) * page 3, line 41 - line 43 * * page 8, line 56 - line 57 * * page 8, line 43 - line 49 *	8,9	
X	EP 0 659 402 A (INDENA SPA) * page 5, line 37 - line 40 * * claims 1,3,6 *	8,9	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	6 November 1997	Polesak, H

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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Application Number
EP 97 11 1635

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	GB 2 280 110 A (HOWARD FOUNDATION) * page 20, line 10 - line 40 * -----	8,9	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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